Vol. XIII. *October, 1907.* *No. 5.*

BIOLOGICAL BULLETIN

THE EARLY DEVELOPMENT OF THE PIGEON'S EGG, WITH ESPECIAL REFERENCE TO THE SUPERNUMERARY SPERM NUCLEI, THE PERIBLAST AND THE GERM WALL.

MARY BLOUNT.

A Preliminary Paper.

In the *American Journal of Anatomy*, September, 1904, there appeared a paper by Dr. E. H. Harper on "The Fertilization and Early Development of the Pigeon's Egg." Dr. Harper found that the egg is polyspermic; that one sperm nucleus unites with the egg nucleus; and that the supernumerary sperm nuclei migrate to the periphery of the germinal area and there set up an accessory cleavage. He followed through the development to the sixteen-cell stage, or about eight hours after fertilization, and although he gives two figures of sections of an egg fifteen hours after fertilization, the intervening stages were not filled in.

At the zoölogical laboratory of the University of Chicago, in January, 1905, I took up the study of the pigeon's egg, hoping to continue from the sixteen-cell stage. But in order to appreciate the material, it was necessary to go back into earlier stages. I have obtained an egg for every hour of development from the formation of polar bodies to the time of laying — a period of about forty-one hours. For some of the more critical stages before laying I have more abundant material, and also have a good many laid eggs.

The purpose of this preliminary paper is to announce some of the more important steps in the early development, but without

presenting the abundant proof which the material affords for my conclusions.

Through the kindness of Prof. C. O. Whitman and other members of the Department of Zoölogy, I have been the recipient of a university fellowship which has enabled me to pursue this study. Dr. F. R. Lillie, at whose suggestion I undertook the research, has followed the work carefully, and I thank him for his interest and kindness. I am also indebted to Mr. W. L. Tower for his help in photography. The living egg is a difficult subject, and it was only after a great many efforts that I secured any photographs. Twelve cleavage stages have been photographed, although only three are presented in this paper.

Methods.

Following the method of workers who have preceded me, the blastoderm has been killed and hardened on the yolk, and the orientation marked with a bristle: Immediately after a window has been made through the shell, a bristle is inserted in the side of the yolk toward the blunt pole of the shell. Later (usually when the egg is in 70 per cent. alcohol) a five-sided piece, including the blastoderm, is cut out from the yolk. One side of the five-sided area is perpendicular to the chalazal axis, and is toward the large pole of the egg. Two sides are parallel to each other and to the chalazal axis, and the last two sides meet in a

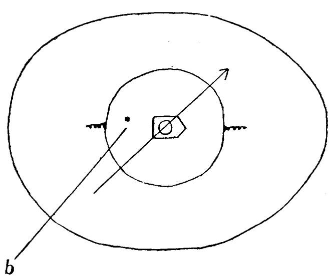

FIG. 1. Diagram to show the method of marking the orientation. The arrow indicates the direction of the axis of the future embryo. *b*, bristle.

sharp angle pointed toward the small pole of the egg. Fig. 1 makes this orientation clear, the anterior side of the blastoderm being toward the point of the arrow. This five-sided block is

easily seen in the paraffin cake for orientation in cutting. Kleinenberg's picro-sulphuric acid (strong solution) plus 10 per cent. acetic has been the most successful for killing and fixing, although other solutions have been used.

Fertilization.

Dr. Harper (3) found that the egg is fertilized in the evening, at the time it leaves the ovarian capsule and enters the oviduct and he makes this statement, "In all cases observed, this has taken place between seven and nine o'clock." I shall, therefore, refer to eight o'clock in the evening as the hour of fertilization, although the exact time for any particular oviducal egg is not known.

To present the history of the early development, I shall describe several critical stages as follows:

1. Position of the supernumerary sperms at the close of maturation.
2. The 8-cell stage.
3. The 16-cell stage.
4. The last stage of the multiplication of the sperm nuclei.
5. The disappearance of the sperm nuclei.
6. The periblast.
7. The growth of the blastodisc at the expense of the periblast.
8. The germ wall.

1. *Position of the Supernumerary Sperm Nuclei at the Close of Maturation.*— In an egg taken from the oviduct at 11:30 P. M., or about three and one half hours after fertilization, the first cleavage plane had not formed. In this egg, the supernumerary nuclei had migrated into the periblast at the periphery of the germinal area, and they occupied a circle which in later stages is indicated superficially by accessory cleavage. Some of these nuclei were in mitotic division.

2. *The 8-cell Stage.* — Abundant material has been obtained in stages of two and four cells, but for brevity, a description of those stages is omitted from this paper.

At 4:45 A. M., eight and three fourths hours after fertilization, an egg of eight (or perhaps nine) cells was taken from the shell gland. Its surface view is shown in Fig. 2, and a transverse

section in Fig. 3. The dotted circle, Fig. 2, represents the distance to which the sperm nuclei may migrate peripherally (it is also the peripheral limit of the periblast nuclei, to be explained later), but the accessory cleavage, with a few exceptions, is con-

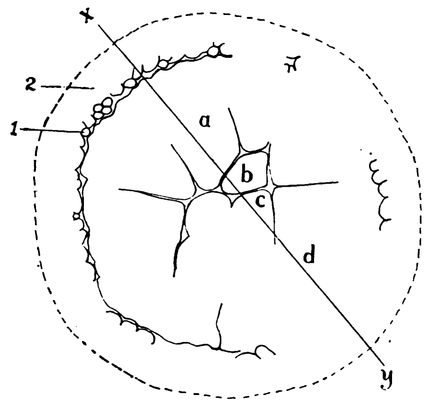

FIG. 2. Sketch of surface view of a pigeon egg about eight and three fourths hours after fertilization, 4:45 A. M. *a*, *b*, *c*, *d*, cells of primary cleavage which are shown in section in Fig. 3. *xy*, the plane of the section in Fig. 3. 1. Accessory cleavage. 2. Periblast.

fined to the zone just outside the blastomeres of the primary area. A migrating sperm nucleus is shown at the extreme right of Fig. 3. Another sperm-nucleus has migrated under the large blastomere *a*. It is in the central periblast, which will be explained later. A study of the whole series of sections showed a number of nuclei in this position, forming a submarginal circle. They migrate later as far centrally as the margins of the nucleus of Pander, but were never found under the very center of the blastoderm.

Fig. 3 is of a section taken through the plane *xy* of Fig. 2.

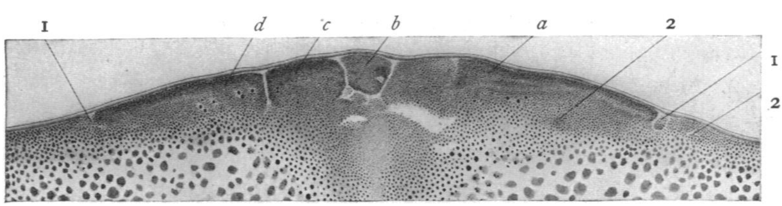

FIG. 3. Transverse section of the pigeon egg whose surface view is shown in Fig. 2. *a*, *b*, *c*, *d*, cells of primary cleavage. 1. Accessory cleavage. 2. Migrating sperm nuclei.

The cells of this section will be recognized as the cells with the corresponding letters in Fig. 2. The small cell *b* is cut off from the underlying yolk in this section, but it is so only at its central end. In sections anterior to this, the cell *b* is continuous with the yolk. The split made by this horizontal cleavage marks the position of the *future segmentation cavity*. This horizontal plane may not be permanently established at this stage. It seems to come and go during the next few hours of development. But *its position indicates the depth of the center of the blastoderm in cleavage stages.*

A comparison of the several stages here represented (Figs. 3, 5, 6 and 9) by actual measurement of the drawings will show that there is but the slightest variation in the depth of the germinal disc at the center.

In contrast with this, is the account of the hen's egg by Kölliker (6). He describes the blastodisc as increasing in depth as cleavage progresses. In his Fig. 19 (which represents a vertical section through a hen's egg of about twenty cells) two central "*Furchungskugeln*" and two marginal "*Segmenten*" are shown; *i. e.*, there are four cells in the section forming a single layer. Between this layer of cells and the white yolk is the unsegmented "*Bildungsdotter.*" None of these products of cleavage is completely cut off from the "*Bildungsdotter.*" They form a layer .14 mm. in depth in the center. A section through a later stage in the development of the hen's egg is shown in Kölliker's Fig. 22 where, "*die Dicke der durchfurchten Stelle in der Mitte des Keimes gerade noch einmal so dick war, als in dem früher beschreibenen Fälle* (*Fig. 19*) *nämlich 0.28–0.30 mm.*" . . . "Somit greift die Durchfurchung, indem sie weiterschreitet, in der Mitte der Keimschicht immer mehr in die Tiefe, wie schon Oellacher dies vermuthet hat, und erreicht am Ende nahezu die Grenze der Lage die in der Fig. 19 mit *bd* als ungefurchten Bildungsdotter bezeichnet ist."

Kölliker suggests that the adding of cells from below may be by a process similar to the adding of cells to the central part from the marginal segments, — *i. e.*, the nucleus of a marginal segment divides and the central end of the segment containing one of the daughter nuclei is cut off and becomes a "Furchungs-

kugel." The other daughter nucleus passes into the marginal segment, and so on until finally the part of the marginal segment left over, changes over into a "Furchungskugel." And so, according to Kölliker, the first appearing *Furchungskugeln* are never completely cut off from the unsegmented *Bildungsdotter* below, but nuclei, sisters to those in the first layer of cells, pass down into the *Bildungsdotter*. Here nuclear division takes place, and cells are organized around the upper daughter nuclei, thus forming the second layer of cells in the center of the blastodisc, while the lower daughter nuclei are left deeper in the "Bildungsdotter." And thus cleavage proceeds downward until finally the last remaining nucleated portions of the "Bildungsdotter" change over into "Furchungskugeln."

In the pigeon's egg, on the contrary, I do not find any such deepening of the center of the blastodisc. The change from one layer to several layers of cells is by a process *exactly like that of the teleost egg*. See Agassiz and Whitman (1) Fig. 2, and Wilson (8) Figs. 16, 17, 18 and 19. *The blastodisc of the pigeon's egg becomes stratified by horizontal cleavage planes arising above the first horizontal cleavage; i. e., above the level of the plane which limits the cell b below* (Fig. 3). *Nuclei are never found in the central part below the level of the horizontal cleavage under the cell b.*

Extending deep into the white yolk is a cone of slightly granular protoplasm. It varies in extent in different stages as will be seen by comparing Figs. 3, 5, 6 and 9. A more central section than Fig. 5 shows this cone extending deeper. In some stages it is better described as being funnel-shaped, with the slender tube of the funnel going deep into the yolk, and the mouth opening on the the lower side of the blastodisc. In some of the sections of the egg represented in Fig. 9 it is found at twice the depth of the figure. If the sections were cut exactly perpendicular to the surface, the funnel would appear continuous from its broad end at the blastodisc to the deepest limit included in the section. A similar structure has been figured by Eycleshymer (2) for the egg of *Lepidosteus osseus*, Figs. 32, 34 and others. He calls it, "the peculiar conical prolongation of the periblast."

3. *The 16-cell Stage.*—Fig. 4 is a photograph of a pigeon egg of sixteen cells. Although of later development, it was obtained an hour earlier than the egg shown in Fig. 2,—*i. e.*, it was taken from the oviduct at 3:45 A. M., seven hours and forty-five minutes after the time from which fertilization is reckoned. The arrow indicates the direction of the axis of the embryo, and the anterior side of the blastoderm is in the direction of the point of the arrow.

Three principal regions in the blastoderm of the bird's egg are to be recognized in surface view at this stage: (1) the *central area*, (2) the *marginal cells*, (3) the *periblast*. In this egg (Fig. 4), the central area is occupied by six cells [the Furchungskugeln of Kölliker (6)] and there are ten marginal cells (Kölliker's Segmenten). The periblast is the zone outside the marginal cells. At the inner margin of this zone is the accessory cleavage caused by the supernumerary sperm nuclei. This is on all sides, but at a few places where there are no sperm nuclei, the large marginal cells are open peripherally.

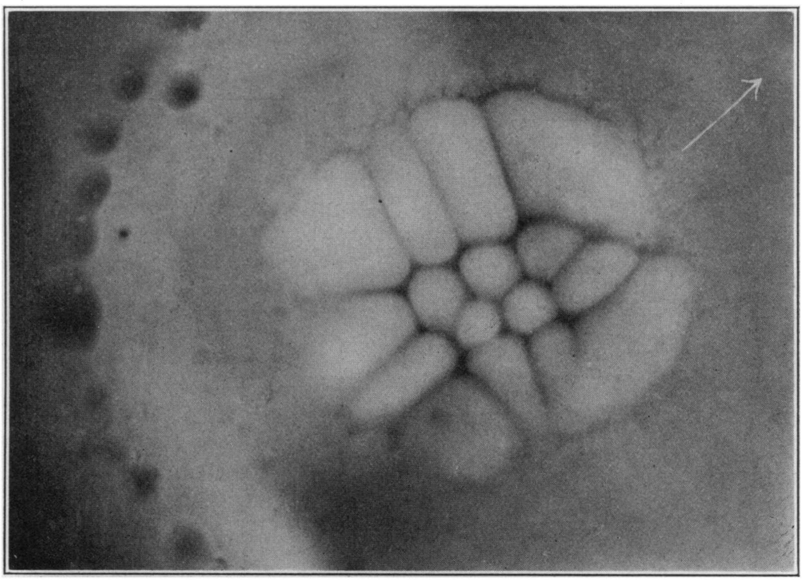

FIG. 4. Photograph of a pigeon's egg 7¾ hours after fertilization. 3.45 A. M. The anterior side of the blastoderm is toward the point of the arrow.

As cleavage proceeds cells are cut off centrally from the marginal cells, and added to the central area, and thus the latter grows at the expense of the former (compare Fig. 7). Radial cleavage planes divide the marginal cells and increase their number, while the central cells are constantly becoming smaller by division. Finally, the marginal cells are all used up, and we recognize only two regions in the blastoderm, (1) the central area, and (2) the periblast. In early stages, all of the cells are continuous with the yolk, but as development proceeds, the central cells become complete below and separate from the yolk, and only the marginal cells are open below. Thus the marginal cells constitute a "zone of junction" (see Agassiz and Whitman (1), Figs. 2, 3, 4 and 5) between the segmented and unsegmented parts of the egg. All of the photographs presented in this paper show a very symmetrical form of cleavage, and while I have found a good many instances of asymmetrical cleavage, I cannot agree with Kölliker (6) that "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der anderen voran ist."

4. *The Last Stage of the Multiplication of the Sperm Nuclei.* — In an egg obtained at 6:30 A. M., ten and a half hours after fertilization, the sperm nuclei were very numerous. There is no record of the exact number of cells of primary cleavage showing

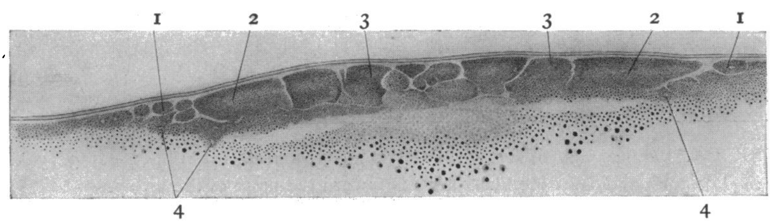

FIG. 5. Transverse section of a pigeon's egg at the end of the period of multiplication of the sperm nuclei. Egg taken 6.30 A. M., about 10 hours after fertilization and 31 hours before laying. Note that all cells are still continuous with the yolk. 1. Accessory cleavage around the sperm nuclei. 2. Marginal cells sharply separated from the sperm nuclei. 3. Central cells. 4. Sperm nuclei.

on the surface of this egg, but there were a few more than thirty-two. The accessory cleavage was very abundant and more than one cell in depth. A transverse section through about the cen-

ter of this blastoderm is shown in Fig. 5. The accessory cleavage is confined to the region immediately outside of the large marginal cells of the blastoderm, but the sperm nuclei have migrated peripherally into the unsegmented part. These nuclei were more abundant than this drawing suggests; for on the right hand side of the section there were four more nuclei in superficial positions in the unsegmented part beyond the limits of the figure. The sperm nuclei were just as abundant in every other section of this egg. But these nuclei have migrated not only peripherally; they are also under the large marginal blastomeres. The latter, however, are definitely separated from the sperm nuclei by cleavage planes whose significance will be better appreciated in contrast with a stage after the disappearance of the sperm nuclei as shown in Figs. 6 and 9.

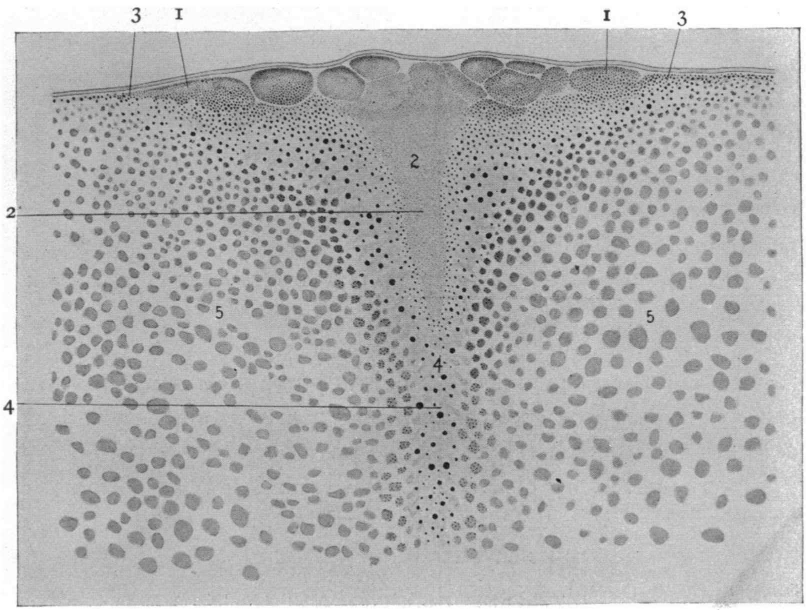

FIG. 6. Longitudinal section of pigeon's egg at the time of disappearance of the sperm nuclei; on the left (anterior), the marginal cell has become open, *i. e.*, continuous with the marginal periblast. On the right the marginal cell is still slightly separated from the periblast at the surface. Surface view of the egg showed traces of accessory cleavage; note continuity of the central cells with central periblast. 1. Marginal cells. 2. Cone of protoplasm. 3. Marginal periblast. 4. Neck of latebra (white yolk). 5. Yellow yolk. Egg taken 7 A. M., about eleven hours from fertilization (estimated).

5. *The Disappearance of the Sperm Nuclei.* — Fig. 6 represents a longitudinal section through about the center of the blastoderm of an egg taken from the oviduct at 7:00 A. M., or eleven hours after the approximate time of fertilization. There were in this egg a few remaining sperm nuclei, and where they occurred, they were separated from the marginal cells by cleavage planes similar to those on the right of Fig. 5. In sections where the sperm nuclei did not appear, the marginal cells were open to the *periblast*, as on the left of Fig. 6. On the right of this figure (which is the posterior side of the blastoderm) the marginal cell is partly closed in, and in a few sections beyond this, it was entirely closed, being separated from a cell of accessory cleavage. In surface view, also, the marginal cells were open peripherally except where the accessory cleavage occurred.

In another egg taken from the bird at 7:00 A. M. (eleven hours from fertilization) *every marginal cell as seen in surface view was open peripherally, and in sections, the margin was like that at the left of Fig. 6. Not one nucleus was found outside the cells of primary cleavage.*

Other eggs of about this period show accessory cleavage on the wane and conditions in sections like those in Fig. 6. In the egg represented in Fig. 5, the sperm nuclei were fragmenting. They disappear between ten and twelve hours after fertilization.

Fig. 7 is a photograph of an egg eleven hours from fertilization (7:10 A. M.). Here, the central, marginal and periblastic regions are clearly expressed. This is probably a stage after the disappearance of the sperm nuclei, and nearly all of the marginal cells are open peripherally. At the posterior side there are suggestions of accessory cleavage. These small cells are probably mere bud-like projections from the periblast, and not due to the presence of sperm nuclei. It is impossible to decide this point from surface view, but sections would show the relations between the marginal cells and the periblast, and therefore demonstrate whether the nuclei of these small cells were derived from supernumerary sperms or from the cleavage nucleus.

6. *The Periblast.*— Previous paragraphs have anticipated the discussion in this. Any mention of the periblast refers the student of vertebrate embryology to the work of Agassiz and Whitman

(1) on *Ctenolabrus* where the origin of the periblast was first accurately described. Only the twelve marginal cells of the sixteen-cell stage of the teleost egg rest upon the yolk. The contact with the yolk is at the inferior outer angle of the cells, and this region "may be designated as the zone of junction" (Agassiz and Whitman) between the blastodisc and the

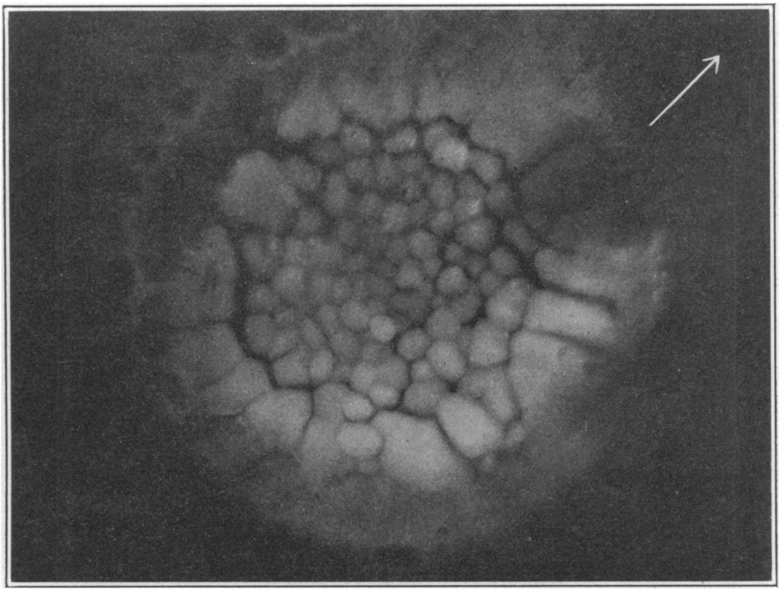

FIG. 7. Photograph of pigeon's egg 11 hours after fertilization, 7.10 A. M. The point of the arrow indicates the anterior side.

periblast. The marginal cells of the teleost are open peripherally. Now, there is to be recognized in the bird's egg a periblast exactly comparable at this stage (eleven or twelve hours after fertilization) with the periblast of the fish egg. We may think of a *potential periblast* in the *unsegmented pigeon's* egg. Into this the sperm nuclei migrate.

After these nuclei disappear the marginal cells of the blastodisc open peripherally to the periblast and are directly continuous beneath with the yolk. The nuclei of the marginal cells divide, and some of the daughter nuclei migrate into the unsegmented region, and thus the periblast "becomes cellular," to use the ex-

pression of Agassiz and Whitman (1). The periblast nuclei migrate peripherally and also into subgerminal positions, and thus we may speak of a *marginal* and *central* periblast. But the nuclei of the central periblast have not been found in the nucleus of Pander.

In *Lepidosteus osseus*, Eycleshymer (2) found the nuclei most numerous at the center, "undergoing rapid division and contributing one derivative to the cell cap.

Fig. 8 is a photograph of an egg obtained at 9:30 A. M., or thirteen and a half hours after fertilization. The marginal cells

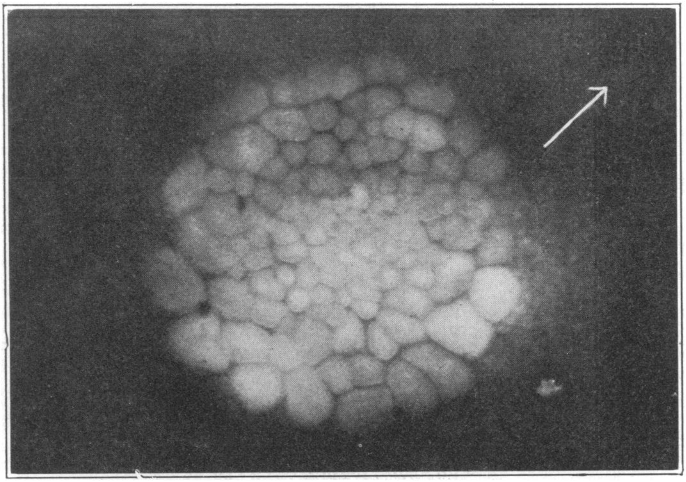

FIG. 8. Photograph of pigeon's egg 13½ hours after fertilization, 9.30 A. M. Anterior side of blastoderm toward point of arrow.

are now limited peripherally, but are open below as is suggested in Fig. 9, a transverse section through another egg of about the same age. The periblast in such an egg as Fig. 8 is demonstrated only in sections. It does not appear in surface view.

7. *The Growth of the Blastodisc at the Expense of the Periblast.* — In the teleost egg, after the conclusion of cleavage, the periblast remains distinct from the blastodisc, but in the pigeon's egg, the periblast continues to add cells to the segmented region. I have studied this point carefully up to several hours after laying, but have not completed my study on later stages of incubation. To illustrate this, there is a series of drawings, Fig. 9 to Fig. 15.

The transverse section represented in Fig. 9 is through about the center of the blastoderm of an egg taken from the bird at 10:30 A. M., or about fourteen and a half hours after fertilization. The center of the blastodisc has become three cells deep, and is separated from the yolk by a sharp line bounding the latter. But the marginal cells are continuous with the yolk, and

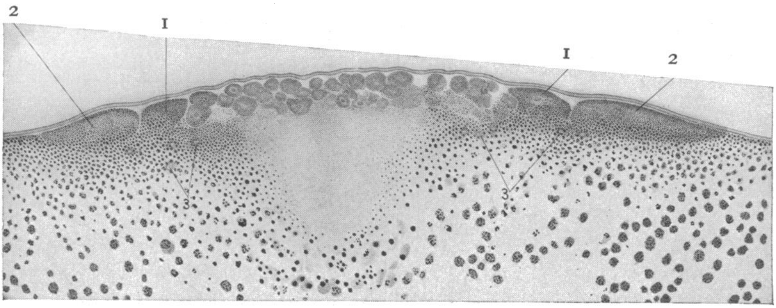

FIG. 9. Transverse section through the center of the blastoderm of a pigeon's egg taken at 10:30 A. M., 14½ hours after fertilization. 1. Marginal cell. 2. Marginal periblast. 3. Nuclei in the central periblast, derived from the nucleus of the marginal cell.

protrusions from the central periblast extend into the segmentation cavity. Nuclei are often found in these protrusions, which suggest that cells are being added to the segmented part. This egg is still in the cleavage stage, being twenty to twenty-two hours before gastrulation [considering the time of gastrulation five to seven hours before laying as determined by Mr. Patterson (7)], and may therefore be considered not unlike the teleost egg. But in following through the successive later stages, similar relations are found between periblast and blastodisc and there is no time when they are distinct.

Fig. 10 shows in mere outline the conditions at the margin of

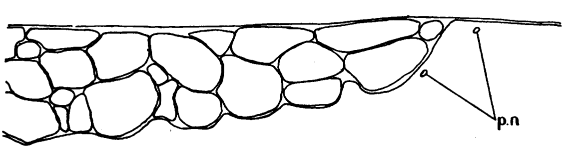

FIG. 10. Margin of a transverse section of a pigeon's egg about twenty and a half hours after fertilization, 4:25 P. M. *p.n*, periblast nuclei.

the blastoderm at 4:25 P. M., or twenty and a half hours after fertilization. Some of these cells at least have been derived by division of such a marginal blastomere as shown in Fig. 9. Others may have been derived from the periblast, with nuclei sisters to those yet remaining in the unsegmented part.

Fig. 11 is the posterior end of a longitudinal section through an egg perhaps twenty-five hours after fertilization (8:50 P. M.) Four nuclear nests and two single nuclei are found in the periblast. Beyond the limits of the drawing, are four other nuclei

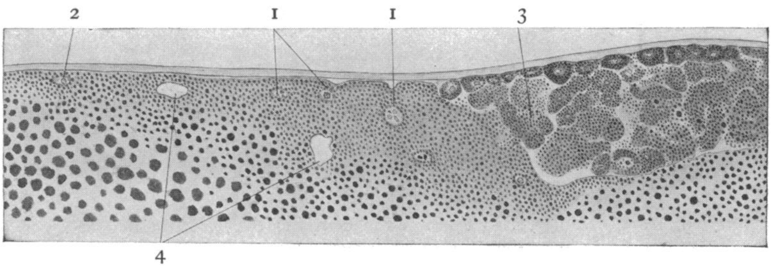

FIG. 11. Posterior side of a longitudinal section of a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Nests of periblast nuclei. 2. Periblast nucleus. 3. Syncytial mass derived from the periblast, organizing into cells which will be added to the blastodisc. 4. Vacuoles.

two of them are in line with the most extreme nucleus to the left and two are a little deeper. Large masses, as shown at 3, Fig. 11, are organized out of the periblast and subsequently they divide into smaller cells. Indentations just to the left of the segmented part here suggest future cleavage which would add superficial cells. (Compare Fig. 14.) This figure (Fig. 11) resembles Harper's (3) Fig. 36 which is a section of an egg fifteen hours after fertilization. Harper considers that the "free nuclei" are sperm nuclei but there was a gap in his material just at the period when the sperm nuclei disappear and the periblast is organized. The nuclei of his Fig. 36 are doubtless periblast nuclei.

Fig. 12 shows a marginal part of a horizontal section through an egg of the same age as Fig. 11 (twenty-five hours after fertilization, 8:50 P. M.). Here are "free nuclei" or periblast nu-

clei and such relations between the segmented and unsegmented part as to suggest contribution of cells from the periblast. Of course, such segregation of cytoplasm and granules around nuclei as is indicated at 2 and 3 may not be permanent. The nucleus for the cell at 3 is in the next section. Amitotic division is suggested by some of the nuclei. Compare the position of

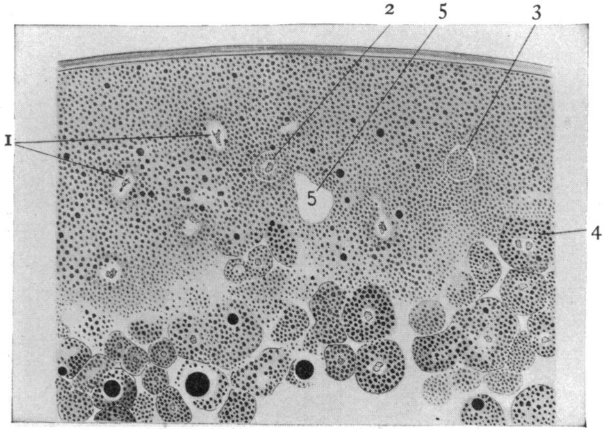

FIG. 12. Marginal part of a horizontal section through a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Periblast nuclei. 2 and 3. Cells being organized out of the syncytium. The nucleus for 3 is in the next section. 4. A cell contributed from the periblast. 5. Vacuole.

nuclei in a syncytial zone outside the segmented blastodisc with the condition shown in Wilson's "Embryology of the Sea Bass" (8), Figs. 23 and 24.

The following outline drawings, Figs. 13, 14, and 15, need

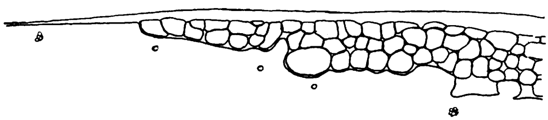

FIG. 13. Margin of a transverse section of a pigeon's egg, about 26 hours after fertilization. Notice cells being added to the segmented part from the periblast. The periblast nuclei were not all in this section, but were found in four successive sections. Two nuclear nests are shown.

little explanation. They are of transverse sections of eggs about 26, 28 and 32 hours respectively after fertilization. They sug-

gest the spreading of the blastoderm over the unsegmented part by cells organized around the superficial periblast nuclei. The blastoderm instead of having an almost perpendicular margin as in Fig. 10, comes to lie over the periblast. These figures show other additions of cells besides those at the extreme margin.

8. *The Germ Wall.*—The term *Keimwall* was first used by His in 1866. In his description of the germ wall of the hen's egg, His (5) says that in the first hours of incubation the white yolk on which the border of the germ area rests is grown through with cells of the germ, and it forms a peculiar structure with protoplasmic frame work enclosing white yolk spheres. To this structure, His gave the name "*Keimwallgewebe*" or *organisirten Keimwall.*

His (5) also says that he was able to follow "wie tiefliegende Zellen des Keimes vermöge ihre sehr ausgesprochenen amöboiden Beweglichkeit die ihnen benachbarten Dotterkörner und Dotterkugeln in sich aufnehmen."

In another paper His (4) says, "Während der ersten Zeit der Bebrütung entsendet die untere Schicht jenes Randtheils Fortsätze zwischen die Elemente des Keimwalles, so dass diese grossentheiles in ein Gerüst archiblastischen Protoplasmas eingeschlossen werden."

This conception of His is based upon a study of hen's eggs during the first few hours of incubation. He makes no reference to the germ wall in the unlaid egg. A study of the pigeon's

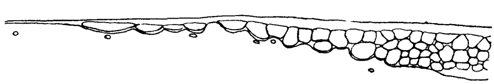

FIG. 14. Margin of transverse section of a pigeon's egg about 28 hours after fertilization. The periblast nuclei, except the most peripheral one, were all in this section.

egg in close stages of development before laying gives quite a different conception of the germwall,—particularly as to the origin of the nuclei. They are *periblast nuclei*, and are not derived from the "tiefliegende Zellen des Keimes." Such nuclei are shown in Fig. 16 which represents the margin of the blastoderm in transverse section of a pigeon egg six hours before laying. It is, of course, a younger stage of the germ wall than is

represented in any of the figures by His (5). Moreover, none of his figures through the germ wall of the hen's egg show the extreme margin of the blastoderm. The nuclei in the unsegmented part in Fig. 16 are periblast nuclei, and their history can be traced back through each preceding stage of development to a period about eleven or twelve hours after fertilization when nuclei from the marginal cells pass into the periblast. Indeed, such a history of the nuclei may be retraced through the figures of this paper — Figs. 16, 15, 14, 13, 11, 10, 9, 6.

As development proceeds from such a stage as is represented in Fig. 9, the zone of junction established by the marginal cells between the blastodisc and the periblast travels outward and the blastodisc increases in diameter as cells are added to its margin

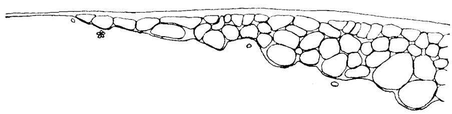

FIG. 15. Margin of a transverse section of pigeon's egg about 32 hours after fertilization. The periblast nuclei were all in this section. One nuclear nest is shown. Notice additions from the periblast to the segmented part.

from the periblast. Cells are organized around the superficial periblast nuclei and sisters to these nuclei are left deeper in the unsegmented periblast. Thus the extreme margin of the blastodisc is thin, Figs. 13, 14 and 15. But later, the deeper sister-nuclei are enclosed in cells and so the blastodisc thickens up under that part which had been only one layer of cells in depth. But, meantime, the *thin margin* has advanced over the yolk, by addition of cells from the periblast. However, there comes a time a few hours before laying (Fig. 16) when the *margin* thickens up. This, I think, is the condition described by His (5), "Am umbebruteten Keim sind die Zellen der unteren Keimschicht von denen der oberen nicht allzusehr verschieden. In dem Randtheil eines unbebrüteten Huhnerkeimes gehen obere und untere Keimschicht in einander über und sie sind nahezu gleich dick. Die untere, lockerer gefügt als die obere, ist eher etwas schwächer. . . . Dotterkörner finden sich auch in Zellen der obern Schicht, obwohl nicht sehr reichlich."

In other literature it is said that the *lower germ layer* forms a

compact mass with the *germ wall*, which, like a thickened border, rests upon the yolk. This thickened border also receives the name *Randwulst* and *bourrelet blastodermique*.

I would describe the margin of the blastoderm (Fig. 16) not as a region where the *upper and under germ layers go over into each other, but as a syncytial region out of which two layers of cells differentiate centrally*.

Through each period of development leading up to this stage (as is shown by the series of figures in this paper), the periblast nuclei keep ahead of the advancing margin of the blastodisc. They multiply and a part of them are used up in the cells that are continually being added to the margin. Such a nucleus in *advance* of the margin of the blastodisc is shown at the left of

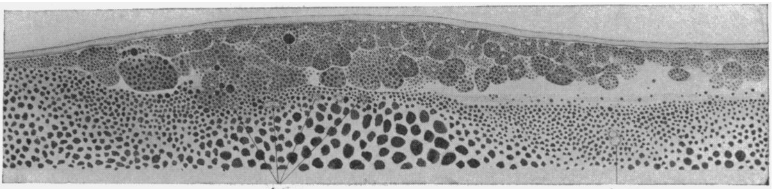

FIG. 16. The germ wall in transverse section through the center of the blastoderm of a pigeon's egg, 8:10 A. M., 36 hours after fertilization and 6 hours before laying. *p.n*, periblast nuclei. They were not all found in this section, but were reconstructed from five successive sections. There are six other periblast nuclei in this half of the section, but in positions central to the limits of the figure. The right hand side of the figure is toward the center of the blastoderm.

Fig. 16. It is in the periblast. It is not enclosed in a cell—*i. e.*, it is not separated by a cleavage plane from the periblast which extends further peripherally — other periblast nuclei are *below* the margin. The thickened-up character of the margin is due to the *upward differentiation* of cells from the periblast. It is not a region where the blastodisc is deepened by the opening of the lower layer of cells to send protoplasmic processes into the white yolk. The cells of this region, which are open below, are so *because they have not yet, in the process of their differentiation out of the periblast, become closed*. Large nucleated masses differentiate *upward* from the periblast. These masses become multinucleate, and finally divide up into several cells. As this region becomes older, that is, as it is left behind while the margin of the

blastoderm advances, the cells individualize and separate from each other. The whole thickened margin is a *syncytium*. It is an *embryonic region* whose depth is measured from the *vitelline membrane* to the lowest limit of the periblast. There is not an ectoderm extending over this region to the extreme margin of the blastoderm. The cells next to the vitelline membrane, are, of course, flattened against it, but their lower border does not give the character of an epithelial layer. Only on the side of this syncytium toward the center of the blastoderm do the cells next the vitelline membrane form a true epithelium. From this thick, syncytial border region cells *individualize centrally*, and form two layers — (1) an upper layer, the ectoderm, and (2) a lower layer of loosely arranged cells.

It is not difficult to explain the presence of yolk-granules in the cells of the upper layer because these cells, like all others in this region are derived from the periblast in which the yolk-granules are abundant.

The section whose margin is shown in Fig. 16 presents two layers of cells in the central part, which roof over a large cavity filled with fluid. The marginal part of the cavity is shown in the figure. Periblast nuclei are everywhere under the blastodisc except in the nucleus of Pander. From them are derived the nuclei which are, in later stages of incubation, in the germ wall of the area opaca (His) and in the Randwulst. I shall not at present attempt to discuss these later stages, but in a more complete paper, I shall present further evidence in support of this conception of the germ wall and shall describe it in other than transverse sections, and shall also describe the formation of the vascular layer.

Summary.

1. The supernumerary sperm nuclei migrate into the potential periblast and disappear between ten and twelve hours after fertilization.

2. The position of the cleavage cavity is indicated by the first horizontal cleavage.

3. After the disappearance of the sperm nuclei, the marginal cells open peripherally and the periblast becomes organized with nuclei derived from the cleavage nucleus.

4. Cells are added to the blastodisc from the marginal and central periblast.

5. The "free nuclei" under the blastodisc are periblast nuclei. They are the nuclei of the germ wall, including the *Randwulst.*

LITERATURE.

1. **Agassiz and Whitman, C. O.**
'84 On the Development of some Pelagic Fish Eggs. Preliminary Notice. Proc. Amer. Acad. Arts and Sciences, XX., 1884.

2. **Eycleshymer, A. C.**
'03 The Early Development of Lepidosteus Osseus. The Decennial Publications, Univ. of Chicago, X., 1903.

3. **Harper, E. H.**
'04 The Fertilization and Early Development of the Pigeon's Egg. Am. Jour. Anat., Vol. III., No. 4, pp. 349–386, Sept., 1904.

4. **His, Wilhelm.**
'75 Der Keimwall des Hühnereies und die Entstehung der parablastischen Zellen 1875. Zeitschrift für An. u. Entwgesch., Bd. I., S. 274 ff.

5. **His, Wilhelm.**
Lecithoblast und Angioblast der Wirbelthiere. Abhandlungen der Königl. Sächs. Gesellschaft der Wissenschaften. Math.-Phys., XXVI.

6. **Kölliker, Albert von.**
'79 Entwicklungsgeschichte des Menschen und der Höheren Thiere. Leipzig, 1879.

7. **Patterson, J. Thos.**
'07 On Gastrulation and the Origin of the Primitive Streak in the Pigeon's Egg. — Preliminary Notice. Biol. Bull., Oct., 1907.

8. **Wilson, H. V.**
'91 The Embryology of the Sea Bass (Serranus Atrarius). Bull. U. S. F. C., Vol. 9, pp. 209–277 (1891).